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Toxicity of Endrin and Malathion Mixtures to Flagfish (*Jordanella floridae*)

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Abstract. Individual endrin and malathion concentrations and mixtures of the concentrations were tested concurrently with flagfish in both chronic and acute exposures. The joint action of mixtures on mortality in chronic exposures consisted of enhanced effects at the higher concentrations not causing death when the pesticides were tested individually. Chronic effects of mixtures on growth followed a simple additive effects model. Malathion did not alter the endrin effects on egg production, and in a separate test it did not affect the uptake or elimination of endrin. Joint action on mortality in acute exposures occurred at 48 and 72 hr but not at 10 and 24 hr.

Persistent toxicant mixtures in the aquatic environment usually occur at sublethal levels; yet, most mixture toxicity studies have been limited to measurements of lethal effects during short-term intervals. Only recently have attempts been made to compare sublethal and lethal or chronic and acute responses to chemical mixtures. Lethal effect (quantal response) concepts and terminology developed by Anderson and Weber (1977) were evaluated for their applicability to sublethal effects by Muska and Weber (1977), and Broderius and Smith (1979) tested the validity of various joint action models with their empirically derived sublethal and lethal effects.

The purposes of this study were: 1) to determine possible differences between the toxicity of individual concentrations and mixtures of them for two different pesticide types—the organophosphate malathion[1] and the chlorinated cyclodiene endrin;[2,3] and 2) to determine if chronic joint toxicity (lethal and sublethal effects) and acute joint toxicity (lethal effects) are the same for a mixture of two insecticides in a fresh-water fish. The experiment was conducted in a system that concurrently exposed fish to individual pesticides and their mixtures. The use of such a system was deemed necessary to avoid differences in biological responses that might occur in consecutive exposures because of different fish stocks or exposure conditions. Thus, the comparison of individual and combined effects in concurrent tests should be more comparable and reliable.

Materials and Methods

Chronic Exposures

Physical System: The exposure system employed was a modification of the one used to expose flagfish to individual concentrations of endrin and malathion in a previous life-cycle experiment (Hermanutz 1978). Two 2-L proportional diluters (Mount and Brungs 1967) were synchronized—one delivered control water and 3 endrin concentrations and the other delivered control water and 3 malathion concentrations. Individual concentra-

[1] Malathion = Diethyl mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate.

[2] Endrin = 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *endo*-5,8-dimethanonaphthalene.

[3] Endrin is a restricted use pesticide. Current allowable uses include treatment of: (1) small grains in Montana for cutworms and grasshoppers, (2) conifer seeds as a bird repellent, (3) cotton for rodents and insects, (4) orchard trees for voles, (5) sugar cane for beetles, and (6) several other uses. Its registration is currently being reexamined.

tion selections were based on data from a previous experiment (Hermanutz 1978), which indicated that one or more of the three concentrations would produce between 25 and 75% reductions on survival or growth or both. Mixtures of these concentrations were attained by drawing proportions of the insecticide dilutions from their respective diluters into mixing chambers located above the site of the test chambers. Flow rates were the same through all chambers. Mixtures of the highest concentrations and the highest combined with middle concentrations were not tested, because such severe effects were expected. Two controls, individual concentrations and mixture concentrations were duplicated and delivered into their respective test chambers. The water supply and treatment, chamber dimensions, lighting systems, photoperiod, embryo incubation apparatus and flow rates were similar to those of the previous experiment.

Chemical System: Methods for metering the acetone-insecticide solutions and the measurements of the insecticides in the water and food were also the same as in the previous experiment. To prevent a gradual decrease in the measured malathion concentration, it was necessary to increase the nominal concentrations by 10% after the eleventh week. As a result, these measured malathion concentrations were less variable than those in the previous experiment. Acetone concentrations ranged from 1.9 mg/L in the lowest concentration to 3.5 mg/L in the highest concentration. Water samples were extracted with petroleum ether and analyzed on a gas chromatograph (GC) with an electron capture detector. GC column specifications were 6 ft length, 4 mm diameter containing Supelcon AW DMCS 100/120 mesh support and 1.5%/1.95% SP2250/SP2401 coating for endrin; and 6 ft, 2 mm column containing Supelcon AW DMCS 80/120 mesh support with 1.5%/1.95% OV17/SP2401 coating for malathion. Mean recovery and standard deviation of the control samples spiked with a known amount of the insecticide was 113 ± 8% (n = 32) for endrin and 89.4 ± 9.6% (n = 31) for malathion. All measured values were adjusted for percent recoveries of the spiked samples. Concentrations were below detection limits of 0.001 µg/L for endrin and 0.01 µg/L for malathion in the control water and below detection limits of 0.01 µg/Kg for endrin and 0.1 µg/Kg for malathion in the fish food.

The mean measured concentrations in the mixtures varied between 95 and 108% of the mean individual endrin concentrations and 99 to 113% of the mean individual malathion concentrations. This was the same variation that was found between duplicates within the same individual concentrations for endrin and slightly greater for malathion (Table 1). Temperatures ranged between 21.7 and 26.8°C for all chambers, and the mean temperature of all chambers ranged between 24.1 and 25.5°C. Dissolved oxygen measurements ranged between 4.7 and 9.6 mg/L for all chambers; mean dissolved oxygen of individual chambers ranged from 7.1 to 7.7 mg/L.

Test water characteristics, which were not altered by the insecticide's presence, were determined weekly by methods described by the American Public Health Association (1971). They were as follows: pH, 6.9–7.8; acidity, 2.0–5.0 mg/L (as $CaCO_3$); alkalinity, 39–45 mg/L (as $CaCO_3$); and total hardness, 43–48 mg/L (as $CaCO_3$).

Biological System: Exposure began by randomly placing thirty 2- and 3-day-old laboratory-cultured flagfish in each of the chambers. All fish came from a common pool. Survival, growth, and spawning assessment procedures were similar to those in the previous experiment. An 84-day spawning period was allowed, but because of periodic substrate removal, the substrates were available for spawning for 33 days. The test was terminated after 140 exposure-days.

Fish were fed three times daily. When less than 30 days-old, they were fed live brine shrimp nauplii; when older, they were fed frozen adult brine shrimp.

Table 1. Measured test concentrations (µg/L) in chronic exposures. Values from duplicates grouped to obtain one mean for each concentration tested

Nominal concentrations[a]	Number of samples	Mean ± S.D.
E. 0.28	21	0.21 ± .03
E. 0.38	19	0.29 ± .04
E. 0.50	21	0.39 ± .04
M 20–22	19	13.8 ± 1.4
M 26–29	22	18.5 ± 2.2
M 35–39	22	23.1 ± 3.1
E/M 0.28/20–22	19	0.20 ± .03/15.3 ± 1.6
E/M 0.28/26–29	18	0.21 ± .03/19.7 ± 1.9
E/M 0.28/35–39	20	0.22 ± .02/23.3 ± 2.8
E/M 0.38/20–22	20	0.28 ± .05/15.6 ± 2.8
E/M 0.38/26–29	21	0.28 ± .06/19.8 ± 2.4
E/M 0.50/20–22	13	0.42 ± .04/13.7 ± 2.2

[a] E = endrin, M = malathion, and E/M = endrin/malathion mixture

Endrin Uptake and Elimination Exposures

Flagfish, initially one-day-old, were exposed for a 15-day uptake and 13-day elimination study by using the chronic exposure system. Reported measured concentration values of endrin are those adjusted for mean recovery of spiked samples. Dissolved oxygen, temperatures, flow rates and other water conditions were similar to those in the chronic exposure experiment. After 15 days, approximately 1/3 of the surviving fish in each concentration were sampled. Immediately after sampling, the toxicant delivery was discontinued, and the diluter continued to deliver uncontaminated lake water for the duration of the test. Additional samples were taken 5 and 13 days after the toxicant delivery was discontinued. Fish from duplicate exposure chambers were combined to obtain larger samples. Sample sizes ranged from 5 to 34 fish, and all fish within a sample were pooled for residue analysis. Residues were based on total-body wet weight; lipid content was not determined. Fish were killed in ice cold water and frozen for later analysis. Residues were extracted and analyzed by gas chromatography according to Food and Drug Administration procedures (U.S. Department of Health, Education and Welfare 1972).

Acute Exposures

Thirty-seven-day-old flagfish were exposed for 168 hr in the same system used for chronic exposures. Forty fish were randomly distributed in each of the duplicate chambers. The test included one duplicated control. Mixtures of the highest concentrations and the highest combined with medium concentrations were not tested. The variation between mixture and individual concentrations (Table 2) was about the same as in the

Table 2. Measured test water concentrations and percent mortality at various time intervals in acute exposures. Values from duplicate test chambers grouped to obtain one mean for each concentration

		Nominal concentrations (μg/L)[a]											
		E	E	E	M	M	M	E/M	E/M	E/M	E/M	E/M	E/M
	Control	.75	.86	.99	265	340	435	.75/265	.86/265	.99/265	.75/340	.86/340	.75/435
		Measured concentrations (μg/L)											
Mean	0	.59	.76	.86	265	331	419	.58/272	.70/278	.96/259	.58/359	.72/360	.54/455
S.D.	—	.02	.10	.15	14	6	13	.12/12	.03/10	.08/17	.07/14	.04/12	.04/21
N	10	6	8	6	5	8	5	10/10	10/10	10/11	10/10	10/10	10/10
Hours		% Mortality[b]											
10	1	1	0	1	29	45	71	36	35	38	58	56	85
24	1	1	0	1	33	56	75	41	36	45	64	66	89
48	1	1	0	1	45	65	83	73	61	73	76	78	91
72	1	3	10	8	64	80	93	90	94	98	88	100	100
96	1	16	41	56	74	89	95	95	99	100	99	100	100
144	3	64	76	93	79	90	98	100	100	100	100	100	100
168	3	85	91	96	79	90	99	100	100	100	100	100	100

[a] E = endrin, M = malathion, and E/M = endrin/malathion mixture
[b] All values rounded off to the nearest integer

chronic exposure. All measured concentration values were adjusted for mean recovery of fortified samples. Temperatures ranged between 23.4 and 24.5°C and dissolved oxygen ranged between 97 and 107% of saturation. Alkalinity, acidity, hardness, and pH of the test water was the same as in the chronic exposure water. Flow rates were about 8 chamber volumes per 24 hr.

Results

Chronic Exposure

Individual Toxicity: The effects of the individual insecticides and their mixtures are presented in Table 3. Endrin survival and growth effects through the first 30 days occurred only at 0.39 μg/L—the highest concentration tested, but adverse effects on the mean number of eggs produced per female occurred in both 0.39 and 0.29 μg/L. None of the three endrin concentrations had any detectable effects on survival or growth beyond 30 days. No adverse effects on survival or egg production were detected for the test duration in any of the three malathion concentrations. The only detectable adverse effect of malathion was the reduction of growth through the first 30 days in 18.5 and 23.1 μg/L (Table 3).

Mixture Toxicity: During the first 30 days, malathion additions of 13.7 to 15.6 μg/L mixed with the various endrin concentrations closely followed the survival curve of the individual endrin concentrations, but malathion additions of 19.7 to 23.3 μg/L produced 22 to 33% higher mortalities than the individual endrin concentrations (Figure 1). Mortality data were tested for independent joint action (Finney 1971) by using the model: $P_{mix} = 1 - (1 - P_e)(1 - P_m)$, where P_{mix} = probability of death in the mixture concentration and P_e and P_m = probability of death in the individual endrin and malathion concentrations. Death probabilities for all individual insecticide concentrations were determined by transforming percent observed mortality to arcsin $\sqrt{\text{percent observed mortality}/100}$ for each of the three concentrations tested and applying these data to a linear regression line where x = concentration in μg/L and y = arcsin $\sqrt{\text{percent observed mortality}/100}$. With a χ^2 test, the expected dead from the independent joint action model compared to the observed dead in 13.7 to 15.6 μg/L of malathion mixed with the endrin concentrations was not significantly different ($p = 0.05$), but the dead observed in 19.7 to 23.3 μg/L of malathion mixed with the endrin was significantly different ($p = 0.05$). Table 4 shows these data transformed back to number of dead fish.

Growth during the first 30 days was slightly reduced in all mixture treatments when mean lengths were compared to mean lengths in the individual insecticides (Table 3). The difference between the control mean length and the mixture's mean length were always 2 to 6% greater than the difference between the control mean length and the smaller mean length of the comparable individual insecticide concentration. From these observations, the reduced growth effects may have been additive so it was tested by applying percent-of-control length (as a decimal fraction) for the natural logarithm of the three concentrations of each individual insecticide to a linear regression line to obtain the ex-

Table 3. Individual and mixture effects on survival, growth and egg production of flagfish in chronic exposure

	Control	Endrin			Malathion		
	0	.39	.29	.21	23.1	18.5	13.8
First 30 days							
Mean % survival	85.0 ± 6.9	23.3* ± 14.1	75.0 ± 25.5	85.0 ± 17.0	86.7 ± 0	90.0 ± 9.9	91.5 ± 2.1
Mean length[a] (mm)	20.9 ± 3.1	17.1* ± 3.8	20.0 ± 3.5	21.3 ± 2.5	18.8* ± 2.6	19.6* ± 2.9	20.0 ± 2.5
% of control	—	82	96	102	90	94	96
Mean eggs spawned per female	1140 ± 278	90* ± 127	556* ± 130	737 ± 119	408 ± 424	1247 ± 281	912 ± 151

	Endrin/Malathion					
	.20/15.3	.21/19.7	.22/23.3	.28/15.6	.28/19.8	.42/13.7
First 30 days						
Mean % survival	81.7 ± 2.3	63.3 ± 14.1	56.7 ± 23.5	70.0 ± 14.1	40.0 ± 9.5	05.0 ± 7.1
Mean length[a] (mm)	19.3 ± 3.7	18.7 ± 3.9	17.5 ± 2.5	18.6 ± 3.7	18.1 ± 3.4	16.7 ± 0.5
% of control	92	89	84	89	87	80
Mean eggs spawned per female[a,b]	921 ± 51	831 ± 166	727 ± 63	541 ± 09	481 ± 200	239 ± 337

* Significantly different from the controls with ANOVA and Dunnett's 1-tailed test ($p = 0.05$). Not applied to mixture concentrations

[a] Mean of replicate tank means

[b] Egg production in the mixtures did not differ significantly (ANOVA, $p = 0.05$) from the equivalent individual endrin concentrations

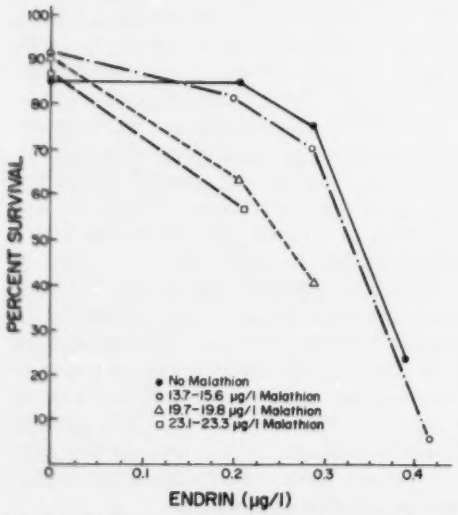

Fig. 1. 30 day survival in endrin with and without malathion additions

pected growth for individual insecticide concentrations. This procedure allowed evaluation of growth as a quasiquantal response. The percentages were added by using the following formula: $P_{mix} = 100 + [(P_e - 100) + (P_m - 100)]$, where P_{mix} = expected percent-of-control length of the mixture and P_e, P_m = expected percent-of-control length for individual endrin and malathion concentrations. These percents were applied to a χ^2 test; the observed values closely agreed with the expected values (Table 5).

None of the mixtures caused detectable survival or growth effects after 30 days. Mixtures did not alter the individual effects on mean number of eggs produced per female; egg production in the mixtures closely followed egg production in the comparable individual endrin concentrations (Table 3).

Endrin Uptake and Elimination

Whole wet-body residues on the 15th exposure day were directly proportional to the water concentration in all individual and mixture concentrations except the individual endrin concentration of 0.29 µg/L. This is shown by the accumulation factors (concentration g/fish/concentration g/exposure water) in Table 6. Eighty-five percent or more endrin residue was lost within 5 days, and 96% or more was lost within 13 days for all individual and mixture concentrations (Table 6). Some of the residue reduction was likely due to growth dilution. Nevertheless, these data show that the presence of malathion had no detectable effects on the uptake or elimination of endrin.

Acute Exposures

All LC_{50}'s were determined by probit analysis (Finney 1971). The three individual malathion concentrations killed the fish so quickly (Table 2) that an LC_{50} could not be determined beyond 48 hr. The 24 and 48 hr LC_{50}'s were calculated at 320 and 280

Table 4. Expected, observed mortality of 60 flagfish on their 30th exposure day in the chronic exposure

Measured concentration (μg/L)	30 day mortality (No. of dead fish)		
	Expected	Observed	χ^2 Contribution
	$Y = -26.4 + 217.7X$[a]		
	$r = .96$		
E.21[e]	6.5[b]	9	0.96
E.29	21.3	15	1.86
E.39	43.4	46	0.16
	$Y = 10.12 + 0.477X$[a]		
	$r = .98$		
M13.8	5.0[b]	5	0.00
M18.5	6.3	6	0.01
M23.1	7.8	8	0.01
	$P_{mix} = [1 - (1 - P_e)(1 - P_m)]100$[c]		
E/M.20/15.3	10.1[d]	11	0.08*
E/M.21/19.7	12.5	22	7.22**
E/M.22/23.3	14.8	26	8.44**
E/M.28/15.6	22.9	18	1.05*
E/M.28/19.8	23.7	36	6.38**
E/M.42/13.7	50.0	57	0.98*
X^2 (.05)2 = 5.99			

* Total χ^2 = 2.11 for 13.7–15.6 μg/L of malathion. Therefore, observed not significantly different from expected
** Total χ^2 = 21.08 for 19.7–23.3 μg/L of malathion. Therefore, observed significantly different from expected
[a] Value in arcsin $\sqrt{\%}$ mortality/100. Value transformed back to percent and divided by 100 for use in $P_{mix} = [1 - (1 - P_e)(1 - P_m)]100$
[b] Expected dead fish obtained by transforming arcsin $\sqrt{\%}$ mortality/100 back to percent and multiplying that value by 60 (number of original fish)
[c] Value in percent
[d] Expected dead fish obtained by multiplying percent by 60 (number of original fish)
[e] E = endrin, M = malathion, E/M = endrin/malathion mixture

Table 5. Expected, observed length of flagfish on their 30th exposure day in the chronic exposure

Measured Concentration (μg/L)	30 day length (% of control)		
	Expected	Observed	χ^2 Contribution
	$Y = (2.75–0.321LnX^b)100$[a]		
E.21[c]	103.4	102	0.02
E.29	93.0	96	0.09
E.39	83.5	82	0.03
	$Y = (1.26–0.144LnX^b)100$[a]		
	$r = -.96$		
M13.8	96.1	96	0.00
M18.5	92.7	94	0.02
	90.2	90	0.00
	$P_{mix} = 100 + (P_e - 100) + (P_m - 100)$[a]		
E/M.20/15.3	99.8	92	0.61
E/M.21/19.7	95.4	89	0.43
E/M.22/23.3	91.9	84	0.68
E/M.28/15.6	88.8	89	0.00
E/M.28/19.8	86.1	87	0.01
E/M.42/13.7	77.3	75	0.07
			$\chi^2(.05)\ 5 = 11.07$
			Total χ^2 of mixtures = 1.79
			Therefore, observed not significantly different from expected

[a] Value in percent
[b] Endrin concentration in ng/L, *e.g.*, E.21 = 210 ng/L, malathion concentration in μg/L
[c] E = endrin, M = malathion, E/M = endrin/malathion mixture

Table 6. Endrin residue accumulation and elimination in larvae-juvenile flagfish

Measured water concentration (μg/L)	No. of fish per sample	Accumulation factor at 15 days	% of 15th day residues eliminated in pesticide free water	
			at 5 days	at 13 days
E[a] .21	24–25	7,900	93	99
E .29	13–24	18,400	95	>99
E .39	22–26	7,100	92	98
E/M .20/15.3	20–21	7,700	92	>99
E/M .21/19.7	12–15	8,100	90	98
E/M .22/23.3	5	7,900	85	97
E/M .28/15.6	13–16	7,800	91	96
E/M .28/19.8	18–20	8,000	lost	97
E/M .42/13.7	13	8,400	85	98
Control	21–24	no residues	—	—

[a] E = endrin, E/M = endrin/malathion mixture

μg/L, respectively. Flagfish reacted so slowly to the three endrin concentrations (Table 2) than an LC_{50} could not be determined before 96 hr. The 96-hr LC_{50} was calculated at 0.85 μg/L. The latest time period that the mixture LC_{50}'s could be compared with individual insecticide LC_{50}'s was 24 hr. After that time, all mixture deaths exceeded 50% (Table 2). A 24-hr LC_{50} was calculated for 300 μg/L malathion mixed with 0.54 to 0.58 μg/L endrin and 315 μg/L malathion mixed with 0.70 to 0.72 μg/L endrin. These LC_{50} values lie so close to the individual malathion 24-hr LC_{50} of 320 μg/L that it is likely that the additions of 0.54 to 0.72 μg/L of endrin had little or no effect on the malathion toxicity during this time interval.

The acute mortality data were tested for independent joint action similar to chronic mortality tests. Because endrin did not show any mortality effects on and before 48 hr, the expected mortality for each of the three concentrations for the 10, 24 and 48 hr periods was estimated to be 0.75%. This was based on a 1% mortality in the control and in 2 of the 3 endrin concentrations (Table 2). The number of dead expected from this model was compared to the observed number of dead with a χ^2 test. The observed dead in the mixture treatments did not differ significantly ($p = 0.05$) from the expected dead at 10 and 24 hr, but they did differ significantly ($p = 0.05$) at 48 and 72 hr. Table 7 shows the expected and observed dead at the critical time periods of 24 and 48 hr.

Discussion

Chronic mortality followed joint independent action in the lower malathion mixture concentrations but not in the higher malathion mixture concentrations, even though all concentrations were below individual chemical mortality thresholds. Individual malathion concentrations, which also were below threshold levels for affecting egg production, did not elicit any discernable mixture effects on egg production. Mortality in the acute test fit the joint independence model during the first 24 hr; subsequently, the mixtures enhanced the individual toxicities. It seems likely that endrin did not begin contributing to the mixture toxicity until 24 hr. This was not demonstrated because the mathematical model deals with death as an endpoint and not the toxic process that leads to it. The variety of individual toxicant effects (or lack of effect) in both the chronic and acute experiments reinforce the observations of Eaton (1973), Muska and Weber (1977), and Broderius and Smith (1979) that meaningful descriptions of joint actions should be made only in relation to the measured response. The results of the present study suggest that concentration (including mixture concentration ratios) and time (exposure duration) must be also considered when describing joint actions.

The additivity of 30 day growth (length) effects, as shown in the model used, appears to fit the concept of "response addition" introduced by Anderson and Weber (1977) and applied to nonquantal (graded) responses by Muska and Weber (1977). The concept, intended to be applied without a precise knowledge of modes of action (Anderson and Weber 1977), retains some identity with the concept of joint independence (Muska and Weber 1977). If the joint independent action model of $P_{mix} = 1 - (1 - P_e)(1 - P_m)$ is applied, with modification where expected individual treatment growth exceeds control growth (all divergences from control growth in the individual insecticides were less than 20%), it produces values similar to the additive effects model. Therefore, the mixture effects on growth may be a result of independent action. But

Table 7. Expected—observed mortality of 80 juvenile flagfish during acute exposure expressed as number of dead fish

Measured concentration (μg/L)	24 hours		
	Expected	Observed	χ^2 Contribution
	Y = .75%		
E 0.59	0.6	1	—
E 0.76	0.6	0	—
E 0.86	0.6	1	—
	$y = -6.39 + 0.160X$[a]		
	r = .99		
M 265	27.7	26	0.10
M 331	42.2	45	0.19
M 419	60.8	60	0.01
	$P_{mix} = [1 - (1 - P_e)(1 - P_e)]100$[b]		
E/M .58/272	29.6	33	0.39
E/M .70/278	30.8	29	0.11
E/M .96/259[c]	26.8	36	—
E/M .58/359	47.3	51	0.29
E/M .72/360	48.9	53	0.34
E/M .54/455[c]	67.3	72	—
		Total χ^2 mixture =	1.13*
		$\chi^2(.05)3$ =	7.81
Measured Concentration (μg/L)	48 Hours		
	Expected	Observed	χ^2 Contribution
	Y = .75%		
E 0.59	0.6	1	—
E 0.76	0.6	0	—
E 0.86	0.6	1	—
	$Y = 2.48 + 0.152X$[a]		
	r = 1.0		
M 265	36.9	36	0.02
M 331	50.7	52	0.03
M 419	67.0	66	0.01
	$P_{mix} = [1(1 = P_e)(1 - P_m)]100$[b]		
E/M .58/272	38.6	58	9.75
E/M .70/278	39.9	49	2.08
E/M .96/259[c]	36.0	58	—
E/M .58/359	56.5	61	0.36
E/M .72/360	56.7	62	0.50
E/M .54/455[c]	72.1	73	—
		Total χ^2 mixture =	12.69**
		$\chi^2(.05)3$ =	7.81

[a] Value in arcsin $\sqrt{\%\ \text{mortality}/100}$. Value transformed back to percent and divided by 100 for use in $P_{mix} = [1(1 - P(1 - P_m)]100$. Expected number of dead fish obtained by multiplying percent value by 80 (number of original fish)

[b] Value in percent dead fish. Expected number of dead fish obtained by multiplying value by 80 (number of original fish)

[c] Beyond linear interpolation because of extreme endrin or malathion concentration

[d] E = endrin, M = malathion, E/M = endrin/malathion mixture

* Observed not significantly different from expected

** Observed significantly different from expected

an assumption of independent action is that the individual toxicants act on different systems, *i.e.*, they have different modes of action (Finney 1971). The toxic effects of malathion and endrin may be different (an acetylcholinesterase enzyme inhibitor and a nerve membrane toxin, respectively) in most animals (O'Brien 1967). However, Sprague (1971) and Muska and Weber (1977) have recognized that often what is considered a mode of action is actually a symptom or an effect. Consequently, it is probably not until all the biochemical processes are resolved that the mode of action is really known. In either case, their effects in this experiment are additive. Muska and Weber (1977), have shown in hypothetical quantal response curves that it may not be possible to distinguish between "response ad-

dition" (mathematically analogous to independent action) and "concentration addition" (mathematically analogous to similar action) at low response levels, as seen in this experiment.

Comparison of individual acute and chronic toxicity values between this experiment and the previous individual pesticides experiment conducted earlier (Hermanutz 1978) ranged from close agreement (endrin acute values) to differences of about a factor of 2 (malathion acute and chronic values). These between-experiment differences are attributed to variability between fish stocks and test procedures, and illustrate the importance of conducting all exposures concurrently in mixtures toxicity tests.

This study has shown that mixture actions between chronic and acute exposures may differ, and that mixture actions within a chronic or acute exposure may differ. Therefore, to determine mixture effects on fish, tests should be conducted for extended time periods at both lethal and sublethal concentrations. In this case, a larva-juvenile exposure duration as suggested by McKim (1977) may have been sufficient to describe the responses in chronic joint toxicity.

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